

# A role of CO and a CO sensor protein in the energy metabolism of *D. vulgaris* Hildenborough

Lara Rajeev, Sergey Stolyar, and David A Stahl, University of Washington  
Grant Zane and Judy Wall, University of Missouri

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Recent studies suggest that carbon monoxide (CO) may play an important role in the energy metabolism of sulfate-reducing bacteria. The membrane bound cytoplasmically oriented CooMKLUXHF hydrogenase of *D. vulgaris* Hildenborough is similar to the Coo hydrogenases of *R. rubrum* and *C. hydrogenoformans*. In the latter systems, the Coo hydrogenases together with the CO dehydrogenase (CODH) oxidize CO to CO<sub>2</sub> and H<sub>2</sub>. Both operons are regulated by a CO sensing transcriptional regulator CooA. The *D. vulgaris* Hildenborough genome also encodes genes for CODH and CooA. Predicted binding sites for CooA are located upstream of both CODH and the Coo hydrogenase operons. To determine if DvH CooA also acts as a CO sensor and if Coo hydrogenase is CO-regulated, we tested a mutant deleted for the *cooA* gene for growth on lactate-sulfate (LS) or pyruvate-sulfate (PS) in the presence of CO. With 1% CO in the headspace, wild type DvH grows efficiently and consumes CO, whereas a *cooA* mutant does not oxidize the CO. Interestingly, the *cooA* mutant grew efficiently on PS with CO in the headspace, but on LS the growth was poor and was inhibited by high H<sub>2</sub> accumulation. The *cooL* mutant lacking an active Coo hydrogenase was able to consume CO. Both wt DvH and the *cooL* mutant, but not the *cooA* mutant, were able to grow on CO as the sole energy source, although growth was very slow. The *cooA* mutant could grow syntrophically on lactate with *M. maripaludis*, whereas the *cooL* mutant could not grow. These observations suggest that the *coo* hydrogenase expression is not CO- or CooA-dependent, unlike that seen in the *R. rubrum* system.

To determine the physiological role of CO in DvH metabolism, we followed the fermentation burst in various DvH hydrogenase mutants. Wt DvH produces very little CO during growth. However, the *cooL* mutant shows a pronounced CO burst during growth on both LS and PS. Other mutants lacking either the Ech hydrogenase or the Hyd (Fe) hydrogenase showed no CO burst, but the *hyd hyn1* double mutant showed a CO burst. Notably, the *cooA* mutant did not accumulate CO during growth. We put forth the hypothesis that Coo and Hyn1 (NiFe) hydrogenases are involved in hydrogen production from lactate and pyruvate, and in the absence of either hydrogenase, electrons from lactate and pyruvate are directed towards CO production. The CO is sensed by